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Dated 31 March 2000

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P01/7700 0.00-0003869.5

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The Patent Office

Cardiff Road
Newport
Gwent NP10 8QQ

1. Your reference — P10281GB

2. Patent application number *(The Patent Office will fill in this part)* — **0003869.5** — 18 FEB 2000

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The University of Bristol
Senate House
Tyndall Avenue
Bristol BS8 1TH

Patents ADP number *(if you know it)* — 4082442001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — Elongase II

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

WITHERS & ROGERS
Goldings House
2 Hays Lane
London
SE1 2HW

Patents ADP number *(if you know it)* — 1776001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

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Novel Polypeptides

The present invention relates to a polyunsaturated fatty acid (PUFA) elongases. More specifically, the invention relates to a DNA sequence from *C. elegans* encoding a PUFA elongase.

Unsaturated fatty acids are essential components required for normal cellular function, being involved in a diverse number of roles ranging from membrane fluidity to acting as signal molecules (Gill, I., Valivety, R. (1997). *Trends Biotechnol.* **15**, 401-409; Broun, P., *et al* (1999) *Ann. Rev. Nutr.* **19**, 197-216). In particular, the class of fatty acids known as the polyunsaturated fatty acids (PUFAs) has attracted considerable interest as pharmaceutical and nutraceutical compounds (Broun *supra*; Horrobin, D. F. (1990) *Reviews in Contemp Pharmacotherpy* **1**, 1-45).

The synthesis of PUFAs i.e. fatty acids of 18 carbons or more in length and containing two or more double bonds, is thought to be catalyzed in a variety of organisms by a specific fatty acid elongase enzyme. This elongase is responsible for the addition of 2 carbon units to an 18 carbon PUFA, resulting in a 20 carbon fatty acid. An example of this reaction is the elongation of γ-linolenic acid (GLA; $18{:}3\Delta^{6,9,12}$) to di-homo-γ-linolenic acid (DHGLA; $20{:}3\Delta^{8,11,14}$) in which the tri-unsaturated 18 carbon fatty acid is elongated by the addition of a two carbon unit to yield the tri-unsaturated 20 carbon fatty acid. Since there is considerable interest in the production of long chain PUFAs of more than 18 carbons in chain length, for example arachidonic acid and eicosapentanoic acid, the identification of this enzyme is of both academic and commercial interest.

At present, there are no examples of identified cloned genes encoding PUFA elongases, though a number of genes encoding enzymes likely to be involved in other aspects of lipid synthesis have been identified. For example, an *Arabidopsis* gene (FAE1) has been shown to be required for the synthesis of very long chain monounsaturated fatty acids (such as erucic acid; $20{:}1\Delta^{11}$) (James *et al*, (1995) *Plant Cell* **7**, 309-319). However, it is clear that this enzyme does not recognize di- and tri-unsaturated 18 carbon fatty acids, for example,

Two related genes were also detected in the genome of *S. cerevisiae*, and their function determined by disruption. These two genes, subsequently named ELO2 and ELO3, were shown to be involved in the elongation of the very long chain saturated fatty acids found in sphingolipid molecules (Oh *et al* (1997), *J. Biol Chem* **272**, 17376-17384). In particular, ELO2 was required for elongation of fatty acids up to 24 carbons, and ELO3 was required for elongation of the 24 carbon fatty acid to 26 carbons. However, neither gene was essential for viability. Examination of the these three fatty acid elongases revealed the presence of a conserved "histidine box" motif (Shanklin *et al.*, (1994), *Biochemistry*, **33**, 12787-12794) (His-X-X-His-His, where X is any amino acid) towards the centre of the polypeptide sequences. Importantly, there was no detectable homology between the yeast elongases (ELO1,2,3) and the plant very long chain mono-unsaturated fatty acid elongase (FAE1) (Oh *et al, supra*).

In order to identify genes encoding PUFA elongases, it is necessary to study systems in which the synthesis of PUFAs is well documented; a good example of this is the model animal system *C. elegans*, a small free-living worm (Tanaka *et al.*, (1996), *Lipids* **31**, 1173-1178). *C. elegans*, like most other animals, and distinct from higher plants, synthesise PUFAs such as arachidonic acid (AA; 20:4 $\Delta^{5,8,11,14}$) as precursors to a class of molecules known as the eicosanoids, which in turn serve as precursors for compounds such as prostaglandins and leucotrienes (Horrobin, (1990), *Reviews in Contemp Pharmacotherpy,* 1:1-45). The presence of AA and other long chain polyunsaturated fatty acids in *C. elegans* is well documented (Tanaka *et al.,* (1996), *Lipids* **31**, 1173-1178). The complete sequence of the nematode's genome is now publically available *(The C. elegans consortium, 1998, Science 282, 2012-2018: Database at http://www.sanger.ac.uk/Projects/C_elgans/blast_server.shtml).*

An object of the invention is to provide an isolated PUFA elongase.

Using the above-mentioned *C. elegans* genomic sequence, together with suitable search strings, the inventors identified eight related putative open reading frames (ORFs) encoding for PUFA elongases. A number of different search criteria were applied to identify a number of (ORFs) which were likely to encode polypeptides with fatty acid elongase

A third aspect of the invention provides a transgenic animal engineered to express a polypeptide according to a first aspect of the invention. The transgenic animal may be engineered to express elevated levels of the polypeptide.

Preferably, the animal is a mammal such as a rat, mouse or monkey. The animal may be a lower eukaryote such as a yeast, or the animal may be a fish.

A fourth aspect of the invention provides a transgenic plant engineered to express a polypeptide according to a first aspect of the invention.

A fifth aspect of the invention provides a PUFA produced by a reaction catalysed by a polypeptide according to a first aspect of the invention.

The PUFA may be di-homo-gamma-linoleic acid ($20:3\Delta^{8,11,14}$), arachidonic acid ($20:4\Delta^{5,8,11,14}$), eicosapentanoic acid ($20:5\Delta^{5,8,11,14,17}$), docosatrienoic acid ($22:3\Delta^{3,16,19}$), docosatetraenoic acid ($22:4\Delta^{7,10,13,16}$), docosapentaenoic acid ($22:5\Delta^{7,10,13,16,19}$) or docosahexaenoic acid ($22:6\Delta^{4,7,10,13,16,19}$).

The PUFA may be a 24 carbon fatty acid with at least 4 double bonds.

The PUFA may be used in foodstuffs, dietry supplements or pharmaceutical compositions.

A sixth aspect of the invention provides a foodstuff comprising a PUFA according to a fourth aspect of the invention. The foodstuff can be fed to an animal.

A seventh aspect of the invention provides a dietry supplement comprising a PUFA according to the fourth aspect of the invention. The dietry supplement can be supplied to an animal to augment its PUFA levels.

An eighth aspect of the invention provides a pharmaceutical composition comprising a polypeptide according to a first aspect of the invention or a PUFA according to a fourth aspect of the invention.

which were identified by this method were then used themselves as search probes, to identify any related *C. elegans* genes which the initial search with the yeast sequences failed to identify. This was necessary because the level of homology between the yeast ELO genes and any worm genes is always low (see BLAST scores later). To allow for a more sensitive search of worm sequences, a novel approach was adopted to circumvent the major drawback with searches using the BLAST programmes, namely that the search string (i.e. the input search motif) must be longer than 15 characters for the algorithm to work. Thus, if it was desired to search for a short motif (like a histidine box), then the BLAST programme would not be capable of doing this. A complete list of all the predicted ORFs present in the *C. elegans* genome exists as a database called Wormpep, which is freely available from the Sanger WWW site (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml). The latest version of Wormpep was down loaded to the hard disc of a Pentium PC, and re-formated as a Microsoft Word6 document, resulting in a document of about 3,500 pages. This was then searched using the "Search & Replace" function of Word6, which also allows for the introduction of "wildcard" characters into the search motif. So, for example, it is possible to search both for the short text string HPGG, which would identify any predicted worm ORF present in the Wormpep 3,500 page document containing this motif, or alternatively search with HPGX (where X is a wild card character). Clearly, such (manual) searches of a 3,500 page document are extremely time-consuming and demanding, also requiring visual inspection of each and every identified ORF. For example, searching with a motif such as HXXHH identifies in excess of 300 different ORFs. However, by using a number of different short search strings (as outlined below), and combining these with other methods for identifying putative elongase enzymes, a number of candidate ORFs have been identified.

Database search using the FAE1 polypeptide sequence

As a negative control, to demonstrate that the FAE1 gene sequence was unlikely to provide a useful search sequence in the identification of *C.elegans* sequences encoding for PUFA elongases, the GenBank databases (http://www.ncbi.nlm.nih.gov/Web/Search/index.html) were searched using the *Arabidopis* FAE1 polypeptide sequence to identify related genes or expressed sequence transcripts (ESTs). GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences (*Nucleic Acid Research* (1998)

The inventors postulated that since fatty acid elongases are expected to be endoplasmic reticulum (ER) membrane proteins, they might be expected to have peptide signals which are responsible for "ER-retention". In the case of ER membrane proteins, this signal often takes the form of a *C*-terminal motif [K-K-$X_{2\text{-}3}$-Stop], or similar variants thereof (Jackson *et al.*, (1990), *EMBO J.*, **9**, 3153-3162). Further sequence analysis of the *C. elegans* putative elongases revealed that 4 ORFs (F41H10.7, F41H10.8, F56H11.4, Y53F4B.c) had *C*-terminal motifs that exactly matched this search pattern, and that a further 2 ORFs (F11E6.5, C40H1.4) had related sequences. These sequence motifs are underlined in SEQ ID 9 to 13, 15 and 16.

Chromosome mapping

Since the inventors had previously observed that *C.elegans* genes involved in the synthesis of PUFA may exist in tandem (for example the Δ5 and Δ6 desaturases required for AA and GLA synthesis, respectively, are < 1 kB apart on chromosome IV (Michaelson *et al.*, (1998), *FEBS Letts* **439**, 215-218), the positions of the putative *C. elegans* elongase ORFs were determined using the Sanger Centre's WebAce *C. elegans* server (http://www.sanger.ac.uk/Projects/C_elegans/webace_front_end.shtml).. This indicated that two pairs of putative elongases were in close proximity to each other on the *C. elegans* chromosome IV.

F41H10.7 and F41H10.8 were identified as being approximately 10 Kb apart on chromosome IV, and F56H11.3 and F56H11.4 were identified as being approximately 2 Kb apart on chromosome IV.

Putative *C. elegans* fatty acid elongases

The positions of the putative ORFs in the*C. elegans* genome are shown below i.e. chromosome number, and map position in centiMorgans, together with the GenBank database accession numbers.

The designations used employ the same method as used on the Sanger Centre's *C. elegans* database, i.e. ORF C40H1.4 is predicted coding sequence 4 on cosmid C40H1.

E (231) > C (226) > G (189) > A (181) > F (166) > D (150) > H (141) > B (140)

Yeast ELO3 (24 to 26 sphingolipid elongase)

D (171) > G(163) > F (154) > A (152) > E (150) > C (131) > B (132) > H (128)

It is clear from the numeric values of the BLAST scores that the sequences are related, but the levels of homology are low. For comparison, the BLAST score for homology between two related worm proteins, the Δ5 and the Δ6 desaturase is in excess of 500.

Analysis of potential sphingolipid ancestry

Previously, the inventors had noted the similarities between the fatty acid Δ6 desaturase and sphingolipid desaturases in plants, and that the two distinct enzymes could have arisen from one ancestral gene. Moreover, it was considered likely that the sphingolipid desaturase predated the fatty acid desaturase, and may in fact have been the ancesteral progenitor. Therefore it is plausible that the next step in the arachidonic acid biosynthetic pathway has also evolved from the sphingolipid metabolic pathway. It is therefore considered highly significant that some of the *C. elegans* ORF putative elongases have similarity to sphingolipid enzymes. For this reason, these ORFs are considered to be very clear candidates for PUFA elongases. It has previously been considered that the *C. elegans* Δ5 and Δ6 fatty acid desaturases have evolved from 1 ancestral gene (Michaelson *et al.*, (1998), *FEBS Letts* **439**, 215-218). It is also significant that one pair of *C. elegans* putative elongase ORFs (F & G) genetically maps close to the Δ5/Δ6 fatty acid desaturase genes, with both gene pairs being located at the top end of chromosome IV.

Cosmid Sanger ID Code	GenBank Acc	Chromosome	Encoded Peptide
W08D2.4	Z70271	IV, 3.06	Δ6 fatty acid desaturase
T13F2.1	Z81122	IV, 3.06	Δ5 fatty acid desaturase

A double construct was also generated by ligating the *Bam*HI/*Xho*I borage Δ^6 insert into the pESC/Δ^5 construct described previously, generating pESC/(Δ^5,Δ^6).

Functional Characterisation in Yeast

Elongases and desaturase constructs were introduced in *Saccharomyces cerevisiae* W303-1A using a lithium acetate based method (Elble, R. (1992) *Biotechniques* **13**, 18-20) and expression of the transgenes was induced by addition of galactose to 2% (w/v) as described in Napier *et al* (Napier, J. A., *et al* (1998) *Biochem J* **330**, 611-614; Michaelson L. V., *supra*; Michaelson, L. V., (1998) *FEBS Letts* **439**, 215-218). Yeast transformants containing pYES2-derived constructs were grown on synthetic minimal media (SD, the composition of which is defined in Sherman, F (1991) *Methods in Enzymology* **194**, 3-21); synthetic minimal medium minus uracil; pESC-derived constructs were grown on SD minimal medium minus tryptophan. Co-transformed yeast (containing both pYES2 and pESC derivatives) were grown on SD minimal medium minus uracil and tryptophan. Prior to induction, cultures were grown in the presence of 2% raffinose and supplemented with 0.5 mM of the appropriate fatty acid substrate in the presence of 1% tergitol-(NP40) (Sigma). All cultures were then grown for a further 48-h unless indicated.

Fatty Acid Analysis

To identify the elongation reaction responsible for the synthesis of di-homo-γ-linolenic acid (DHGLA; 20:3$\Delta^{8,11,14}$) from GLA, this latter fatty acid was supplied as the (exogenous) substrate.

Lipids were extracted from leaves of transformed and control yeast by homogenisation in MeOH-$CHCl_3$ using a modification of the method of Bligh and Dyer (Dickenson & Lester (1999) *Biochim Biophys Acta* **1426**, 347-357). The resulting $CHCl_3$ phase was evaporated to dryness under nitrogen gas and the samples were transmethylated with 1M HCl in methanol at 80 °C for 1 hour. Fatty acid methyl esters (FAMES) were extracted in hexane and purified using a small column packed with Florisil. Analysis of FAMES was conducted using a Hewlett Packard 5880A Series Gas Chromatograph equipped with a

gene present on chromosome IV (present on BAC clone B207d4; AC004050). The GenBank accession numbers are given for all sequences.

The range of fatty acids synthesised by *C. elegans* can potentially require a number of different elongation reactions (Tanaka, T., (1996) *Lipids* 31, 1173-1178). The substrate-specificity of the F56H11.4 PUFA elongase was therefore determined using a range of exogenously supplied fatty acids. This revealed that GLA is the major substrate, with a number of other fatty acids being elongated at a lower efficiency (see Table 1). Although most of these substrates are polyunsaturated fatty acids, it was unexpectedly observed that palmitoleoic acid (PA; 16:1 Δ^9) was also elongated by F56H11.4 to yield vaccenic acid (VA; 18:1 Δ^{11}). The biosynthetic pathway for VA is unclear, but the data indicate that it may be synthesised by elongation of Δ^9-monounsaturated 16C fatty acid.

The *C. elegans* PUFA elongase ORF F56H11.4 maps to the top of chromosome IV (at 4.32 cM) with a related sequence (F56H11.3; 51 % similarity) located 1,824bp downstream. Another *C. elegans* gene (F41H10.8) was also observed, which is present on chromosome IV, and which shows a slightly higher level (53%) of similarity to the PUFA elongase than F56H11.3 (see Fig. 10). However, when a PCR product encoding ORF F41H10.8 was expressed in yeast in a manner identical to that used for F56H11.4, the former failed to direct the elongation of any fatty acids, despite the provision of a range of substrates (see Table II).

In order to reconstitute the PUFA biosynthetic pathway in a heterologous system, the PUFA elongase F56H11.4 was expressed in yeast in conjunction with either the Δ^6- or Δ^5-fatty acid desaturases previously isolated and characterised by the inventors (Napier, J. A., *supra*; Michaelson, L. V., *supra*). Expression of the Δ^6-fatty acid desaturase and F56H11.4 was carried out in the presence of two different substrates (LA or ALA) while the Δ^5-fatty acid desaturase and the elongase were expressed in the presence of GLA only. This demonstrated that was possible to combine a desaturase and an elongase in yeast to generate significant amounts of a final "product" (see Table III). In the case of the elongase and the Δ^6-fatty acid desaturase, the reactions proved highly efficient with the production of 4.5% of DHGLA from the LA substrate. This resulted from 25%

Peak identification and confirmation were carried out by GC-MS using a Kratos MS80RFA using known standards (Sigma). The identity of this 20C PUFA was verified by GCMS, indicating that the conversion efficiency from LA was 0.65%. When ALA was used as a substrate, 12.5% of the (Δ^6-desaturated and elongated) eicosatetraenoic *n-3* fatty acid was Δ^5-desaturated, resulting in a total conversion of 0.3% of the ALA substrate to EPA (the identity of EPA was confirmed by GCMS).

Expression of *C. elegans* elongase in plants

In order to express *C. elegans* elongase in plants, the following protocol can be used to create the transgenic plants. *C. elegans* ORF sequence can be subcloned into a plant expression vector pJD330, which comprises a viral 35S promoter, and a Nos terminator. The resulting cassette or promoter/coding sequence/terminator can then be subcloned into the plant binary transformation vector pBin 19, and the resulting plasmid introduced into *Agrobacterium tumefaciens*. This *Agrobacterium* strain can then be used to transform Arabidopsis by the vacuum-infiltration of inflorescences, and the seeds harvested and plated onto selective media containing kanamycin. Since pBin 19 confers resistance to this antibotic, only transformed plant material will grow. Resistant lines can therefore be identified and self-fertilized to produce homozygous material. Leaf material can then be analyzed for expression of *C. elegans* elongase.

Fatty acid methyl ester analysis can be carried out as previously described.

Table II

	mole% Fatty Acids									
ORF	F41H10.8									
Substrate	-		GLA		LA		ALA		EPA	
Induction	+	-	+	-	+	-	+	-	+	-
16:0	19.0 ± 0.9	19.3 ± 0.2	28.1 ± 0.6	28.0 ± 0.9	23.9 ± 0.7	24.4 ± 0.2	22.8 ± 0.2	23.4 ± 0.2	23.0 ± 0.6	23.7 ± 0.9
16:1	50.9 ± 0.7	50.8 ± 0.6	33.5 ± 2.2	35.5 ± 1.5	22.4 ± 2.1	23.6 ± 0.3	17.6 ± 0.2	15.8 ± 0.9	34.7 ± 3.6	32.2 ± 3.2
18:0	4.2 ± 0.1	5.1 ± 0.1	5.3 ± 0.1	5.6 ± 0.1	5.1 ± 0.2	5.8 ± 0.1	5.4 ± 0.3	5.9 ± 0.1	4.8 ± 0.7	5.1 ± 0.3
18:1	24.5 ± 1.3	24.9 ± 0.5	16.2 ± 1.4	17.1 ± 1.0	9.1 ± 0.3	10.1 ± 0.2	7.8 ± 0.1	9.5 ± 0.6	15.3 ± 2.5	15.3 ± 1.8
18:1*	ND	-	ND	-	ND	-	ND	-	ND	-
LA	-	-	-	-	39.5 ±0.6	36.1 ± 0.4	-	-	-	-
ALA	-	-	-	-	-	-	46.4 ± 0.5	45.4 ± 1.3	-	-
GLA	-	-	14.3 ± 1.6	14.2 ± 0.6	-	-	-	-	-	-
20:2	-	-	-	-	ND	-	-	-	-	-
DHGLA	-	-	ND	-	-	-	-	-	-	-
20:3	-	-	-	-	-	-	ND	-	-	-
EPA	-	-	-	-	-	-	-	-	22.3 ± 2.8	23.8 ± 2.2
% Elongated										
GLA	-	-	0	-	-	-	-	-	-	-
LA	-	-	-	-	0	-	-	-	-	-
ALA	-	-	-	-	-	-	0	-	-	-
EPA	-	-	-	-	-	-	-	-	0	-

19

SEQ ID1

C40H1.4

atggagcttgccgagttctggaatgatctcaacaccttcaccatctacggaccgaatcac
acagatatgaccacaaaatacaaatattcatatcacttcccaggtgaacaggtggcggat
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SEQ ID2

D2024.3

atggcaaaatacgactacaatccgaagtatgggttagaaaattacagcatattccttccc
tttgagacatcttttgatgcatttcgatcgacaacatggatgcaaaatcactggtatcaa
tcaattacagcatctgtcgtgtatgtagccgtcattttacaggaaagaaggtggttctc
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ggttctgcct tctcatggaa atctcttatg tcgttctgtt cggaaacttc
tactatcaat catacatcaa gggaggtggc aagaagttta atgcagagaa
gaagactgaa aagaaaattg aataa

SEQ ID6

F56H11.3

atgtatttgaattatttcgcgacggaaatcttccatcgtagtgcggtttgtgaaacagaa
gcttgtcgctcgtcaaaaataatgattgctgacgtgttcaaatggaaattcgatgcaaac
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ctttga

SEQ ID7

F56H11.4 (Ce 166)

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251 MVMGVIIGVT VYRIKSSGEY CQQTWDNLGL CFGVYFTYFL LFANFFYHAY
301 VKKNNRTVNY ENNSKNFPDL VLIYLRKKVS RKSKNRQCSE NNYKIQFSSN
351 FVNVDGKKHK KTYELILPRR KMTTILTFLF GKNRIFSKYQ KNRKNISIPV
401 DFEILEPKED INANIAEPSI TTRSAAARRK VQKAD*

SEQ ID11

C

1 MAAAQTSPAA TLVDVLTKPW SLDQTDSYMS TFVPLSYKIM IGYLVTIYFG
51 QKLMAHRKPF DLQNTLALWN FGFSLFSGIA AYKLIPELFG VFMKDGFVAS
101 YCQNENYYTD ASTGFWGWAF VMSKAPELGD TMFLVLRKKP VIFM**HWYHHA**
151 LTFVYAVVTY SEHQAWARWS LALNLAVHTV MYFYFAVRAL NIQTPRPVAK
201 FITTIQIVQF VISCYIFGHL VFIKSADSVP GCAVSWNVLS IGGLMYISYL
251 FLFAKFFYKA YIQKRSPTKT SKQE*

SEQ ID12

D

1 MSSDDRGTRT FKMMDQILGT NFTYEGAKEV ARGLEGFSAK LAVGYIATIF
51 GLKYYMKDRK AFDLSTPLNI WNGILSTFSL LGFLFTFPTL LSVIRKDGFS
101 HTYSHVSELY TDSTSGYWIF LWVISKIPEL LDTVFIVLRK RPLIFM**HWYH**
151 HALTGYYALV CYHEDAVHMV WVVWMNYIIH AFMYGYYLLK SLKVPIPPSV
201 AQAITTSQMV QFAVAIFAQV HVSYKHYVEG VEGLAYSFRG TAIGFFMLTT
251 YFYLWIQFYK EHYLKNGGKK YNLAKDQAKT QTKKAN*

SEQ ID13

E

MPQGEVSFFE VLTTAPFSHE LSKKHIAQTQ YAAFWISMAY VVVIFGLKAV
MTNRKPFDLT GPLNLWNAGL AIFSTLGSLA TTFGLLHEFF SRGFFESYIH
IGDFYNGLSG MFTWLFVLSK VAEFGDTLFI ILRKKPLMFL **HWYHH**VLTMN
YAFMSFEANL GFNTWITWMN FSVHSIMYGY YMLRSFGVKV PAWIAKNITT
MQILQFVITH FILFHVGYLA VTGQSVDSTP GYYWFCLLME ISYVVLFGNF
YYQSYIKGGG KKFNAEKKTE KKIE*

SEQ ID14

F

1 MYLNYFATEI FHRSAVCETE ACRSSKIMIA DVFKWKFDAN ELWSLLTNQD
51 EVFPHIRARR FIQEHFGLFV QMAIAYVILV FSIKRFMRDR EPFQLTTALR
101 LWNFFLSVFS IYGSWTMFPF MVQQIRLYGL YGCGCEALSN LPSQAEYWLF

Claims

1. An isolated polypeptide comprising a functional long chain polyunsaturated fatty acid (PUFA) elongase.

2. A polypeptide according to claim 1 wherein the polypeptide extends the chain length of an 18 carbon PUFA to 20 carbons in length.

3. A polypeptide according to claim 1 or claim 2 wherein the polypeptide has at least a portion of the amino acid sequence shown in SEQ ID 15, or variants thereof.

4. A polypeptide according to any of claims 1 to 3 wherein the polypeptide is from an animal.

5. A polypeptide according to claim 4 wherein the animal is an invertebrate.

6. A polypeptide according to claim 5 wherein the invertebrate is a worm.

7. A polypeptide according to claim 6 wherein the worm is *C. elegans*.

8. A polypeptide according to claim 4 wherein the animal is a vertebrate.

9. A polypeptide according to claim 8 wherein the vertebrate is a mammal.

10. A polypeptide according to claim 9 wherein the mammal is a human, rat or mouse.

11. An isolated DNA sequence encoding a polypeptide according to any preceding claim.

12. A DNA sequence according to claim 11 wherein the cDNA comprises the sequence shown in SEQ ID 7 or variants of that sequence due to base substitutions, deletions and/or additions.

25. A pharmaceutical composition comprising a polypeptide according to any of claims 1 to 10.

26. A pharmaceutical composition comprising a PUFA according to any of claims 20 to 22.

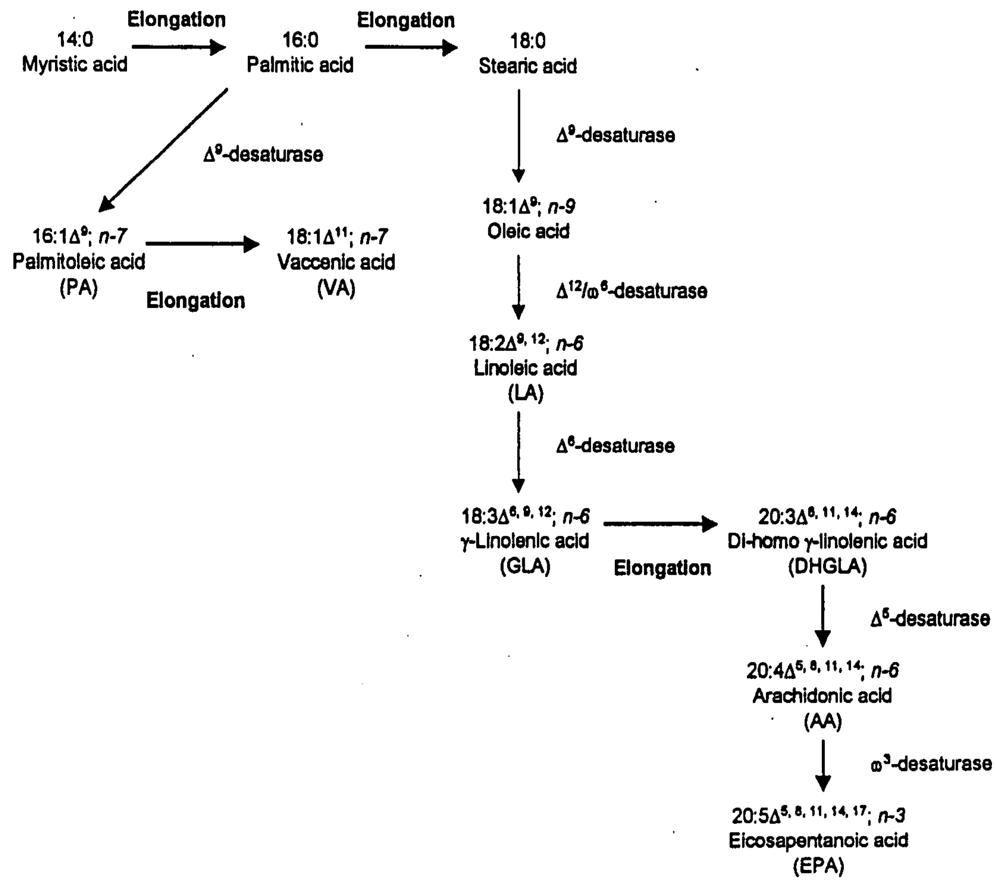
27. A pharmaceutical composition according to claim 25 or claim 26 wherein the composition comprises a pharmaceutically-acceptable diluent, carrier, excipient or extender.

28. A method of elevating the PUFA levels of an animal or a plant by supplying to the animal or plant a polypeptide according to any of claims 1 to 10, a DNA sequence according to claim 11 or claim 12, a foodstuff according to claim 23, a dietry supplement according to claim 24, or a pharmaceutical composition according to any of claims 25 to 27.

29. A method of treatment according to claim 27 wherein the animal is a mammal.

30. A method of treatment according to claim 28 wherein the mammal is a human.

FIG. 1


14:0
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Elongation
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Palmitic acid
Elongation
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Stearic acid
Δ9-desaturase
Δ9-desaturase
16:1Δ9; n-7
Palmitoleic acid
(PA)
Elongation
18:1Δ11; n-7
Vaccenic acid
(VA)
18:1Δ9; n-9
Oleic acid
Δ12/ω6-desaturase
18:2Δ9, 12; n-6
Linoleic acid
(LA)
Δ6-desaturase
18:3Δ6, 9, 12; n-6
γ-Linolenic acid
(GLA)
Elongation
20:3Δ8, 11, 14; n-6
Di-homo γ-linolenic acid
(DHGLA)
Δ5-desaturase
20:4Δ5, 8, 11, 14; n-6
Arachidonic acid
(AA)
ω3-desaturase
20:5Δ5, 8, 11, 14, 17; n-3
Eicosapentanoic acid
(EPA)

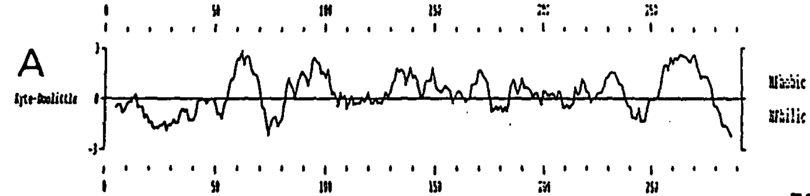


FIG 2

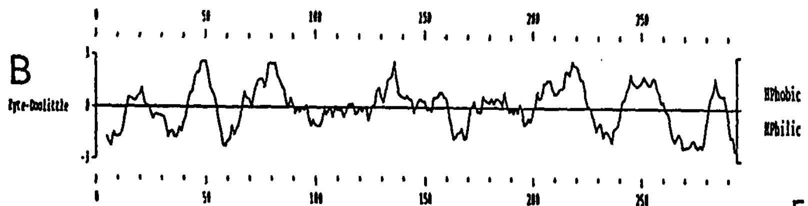


FIG 3

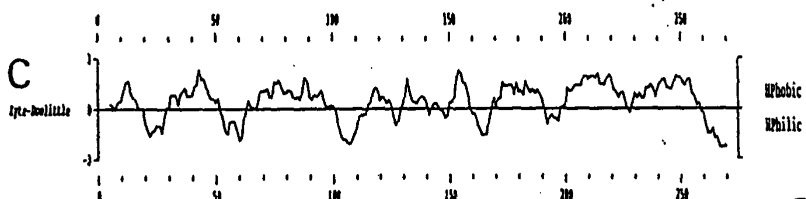


FIG 4

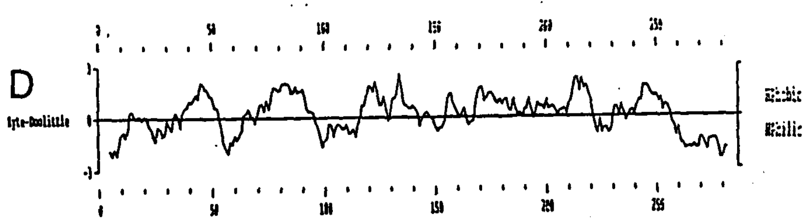


FIG 5

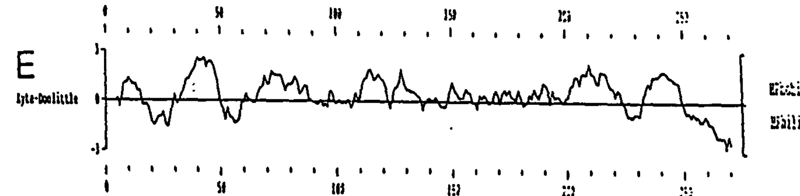


FIG 6

FIG 7

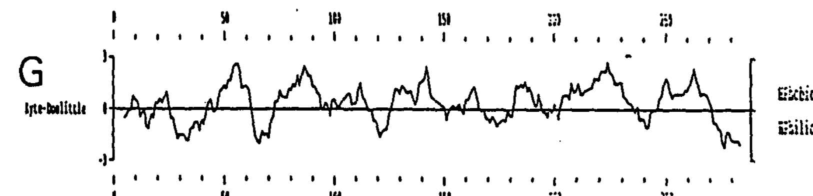


FIG 8

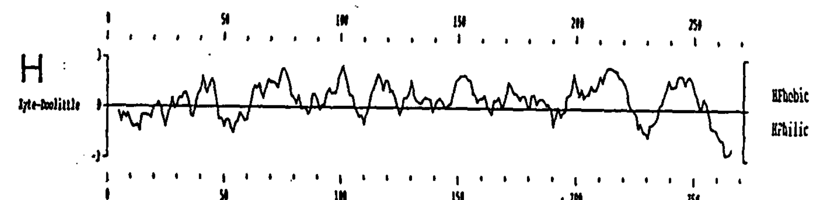
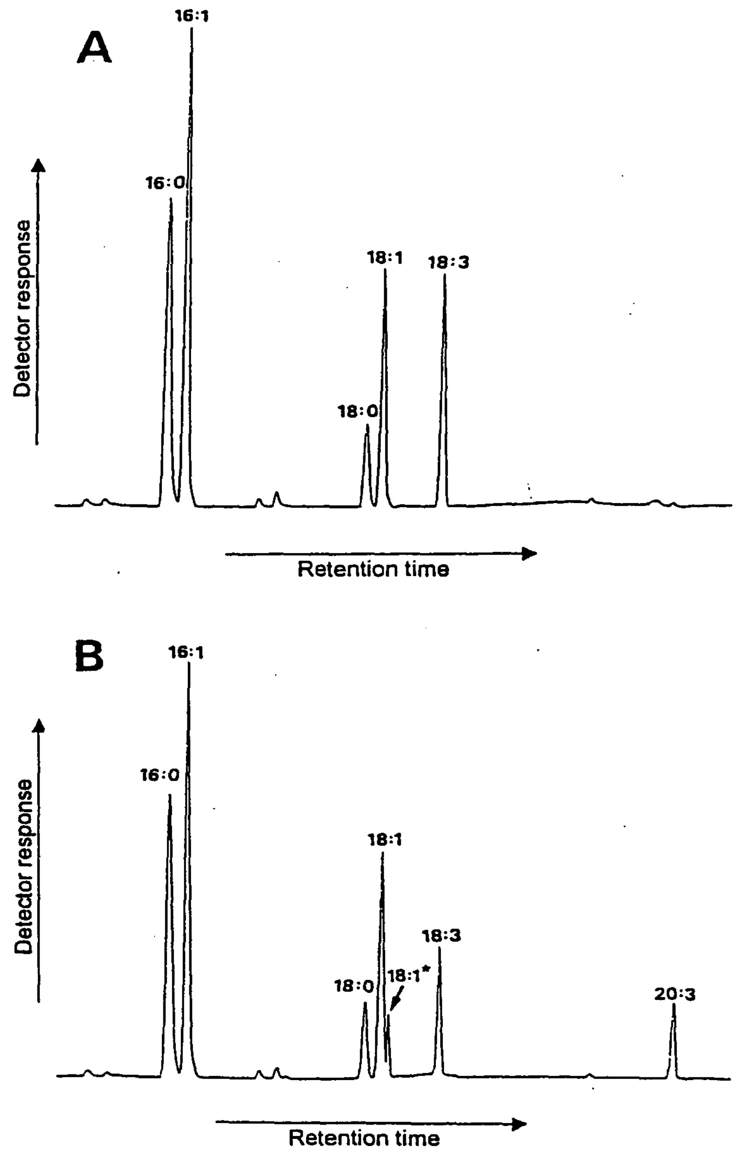


FIG 9

FIG. 10

```
Elo1      -----------MVSDWKNFCLEKASR---FRPTIDRPFFNIYLWDYFNRAVGWATAGRFQ
Elo2      --------MNSLVTQYAAPLFERYPQLHDYLPTLERPFFNISLWEHFDDVVTRVTNGRFV
Elo3      MNTTTSTVIAAVADQFQSLNSSSSCFLKVHVPSIENP-FGIELWPIFSKVFEYFSG--YP
Cig30     -------------------------------------------MDTSMNFSRGLKMDLMQ
B207d4    ------------------------------------------------------------
F56H11.4  ---------------------------------MAQHPLVQRLLDVKFDTKRFVAIATHG
F41H10.8  ---------------------------------------------MPQGEVSFFEVLTTA
F56H11.3  -----------MYLNYFATEIFHRSAVCETEACRSSKIMIADVFKWKFDANELWSLLTNQ

Elo1      PKDFEFTVGKQPLSEPR--PVLLFIAMYYVVIFGGRSLVK--SCKPLKLRFISQVHNLML
Elo2      PSEFQFIAGELPLSTLP--PVLYAITAYYVIIFGGRFLLS--KSKPFKLNGLFQLHNLVL
Elo3      AEQFEFIHNKTFLANGY--HAVSIIIVYYIIIFGGQAILRALNASPLKFKLLFEIHNLFL
Cig30     PYDFETFQDLRPFLEEYWVSSFLIVVVYLLLIVVGQTYMR--TRKSFSLQRPLILWSFFL
B207d4    ------------------------------------------------------------
F56H11.4  PKNFPDAEG-RKFFADHFDVTIQASILYMVVVFGTKWFMR--NRQPFQLTIPLNIWNFIL
F41H10.8  P--FSHELS-KKHIAQTQYAAFWISMAYVVVIFGLKAVMT--NRKPFDLTGPLNLWNAGL
F56H11.3  DEVFPHIR-ARRFIQEHFGLFVQMAIAYVILVFSIKRFMR--DREPFQLTTALRLWNFFL

Elo1      TSVSFLWLILMVEQMLPIVYRHGLYFAVCNVESWTQPMETLY-YLNYMTKFVEFADTVLM
Elo2      TSLSLTLLLLMVEQLVPIIVQHGLYFAICNIGAWTQPLVTLY-YMNYIVKFIEFIDTFFL
Elo3      TSISLVLWLLMLEQLVPMVYHNGLFWSICSKEAFAPKLVTLY-YLNYLTKFVELIDTVFL
Cig30     AIFSILGTLRMWKFMATVMFTVGLKQTVCFAIYTDDAVVRFWSFLFLLSKVVELGDTAFI
B207d4    -------------------------------------------------------DTIFI
F56H11.4  AAFSIAGAVKMTPEFFGTIANKGIVASYCKVFDFTKGENGYWVWLFMASKLFELVDTIFL
F41H10.8  AIFSTLGSLATTFGLLHEFFSRGFFESYIHIGDFYNGLSGMFTWLFVLSKVAEFGDTLFI
F56H11.3  SVFSIYGSWTMFPFMVQQIRLYGLYGCGCEALSNLPSQAEYWLFLTILSKAVEFVDTFFL

Elo1      VLKHRKLTFLHTYHHGATALLCYNQLVGYTAVTWVPVTLNLAVHVLMYWYYFLSASGIRV
Elo2      VLKHKKLTFLHTYHHGATALLCYTQLMGTTSISWVPISLNLGVHVVMYWYYFLAARGIRV
Elo3      VLRRKKLLFLHTYHHGATALLCYTQLIGRTSVEWVVILLNLGVHVIMYWYYFLSSCGIRV
Cig30     ILRKRPLIFVHWYHHSTVLLFTSFGYKNKVPSGGWFMTMNFGVHSVMYTYYTMKAAKLKH
B207d4    ILRKQKLIFLHWYHHITVLLYSWYSYKDMVAGGGWFMTMNYGVHAVMYSYYALRAAGFRV
F56H11.4  VLRKRPLMFLHWYHHILTMIYAWYSHPLTPGFNRYGIYLNFVVHAFMYSYYFLRSMKIRV
F41H10.8  ILRKKPLMFLHWYHHVLTMNYAFMSFEANLGFNTWITWMNFSVHSIMYGYYMLRSFGVKV
F56H11.3  VLRKKPLIFLHWYHHMATFVFFCSNYPTPSSQSRVGVIVNLFVHAFMYPYYFTRSMNIKV

Elo1      --WWKAWVTRLQIVQFMLDLIVVYYVLYQKIVAAYFKNACTPQCEDCLGSMTAIAAGAAI
Elo2      --WWKEWVTRFQIIQFVLDIGFIYFAVYQKAVHLYF--PILPHCGDCVGSTTATFAGCAI
Elo3      --WWKQWVTRFQIIQFLIDLVFVYFATYTFYAHKYL-DGILPNKGTCYGTQAAAAYGYLI
Cig30     PNLLPMVITSLQILQMVLGT-IFG-IL------NYIW---RQEKGCHTTTEHFFW-SFML
B207d4    SRKFAMFITLSQITQMLMGC-VVN-YL------VFCW---MQHDQCHSHFQNIFW-SSLM
F56H11.4  PGFIAQAITSLQIVQFIISCAVLA-HL------GYLM---HFTNANCDFEPSVFKLAVFM
F41H10.8  PAWIAKNITTMQILQFVITHFILF-HV------GYLA----VTGQSVDSTPGYYWFCLLM
F56H11.3  PAKISMAVTVLQLTQFM--CFIYGCTL------MYYS---LATNQARYPSNTPATLQCLS

Elo1      LTSYLFLFISFYIEVYKRGSASGKKKINKNN-----------------------------
Elo2      ISSYLVLFISFYINVYKRKGTKTSRVVKRAHGGVAAKVNEYVNVDLKNVPTPSPSPKPQH
Elo3      LTSYLLLFISFYIQSYKKGGKKTVKKESEVSGSVASGSSTGVKTSNTKVSSRKA------
Cig30     YGTYFILFAHFFHRAYLRPKGKVASKSQ--------------------------------
B207d4    YLSYLVLFCHFFFEAYI-------------------------------------------
F56H11.4  DTTYLALFVNFFLQSYVLRGGKDKYKAVPKKKNN--------------------------
F41H10.8  EISYVVLFGNFYYQSYIKGGGK-KFNAEKKTEKKIE------------------------
F56H11.3  YTLHLL------------------------------------------------------

Elo1      ----
Elo2      RRKR
Elo3      ----
Cig30     ----
B207d4    ----
F56H11.4  ----
F41H10.8  ----
F56H11.3  ----
```

FIG. 11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16:0
18:1
18:3
18:0
Detector response
Retention time
B
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16:0
18:1
18:3
18:0
18:1*
20:3
Detector response
Retention time